

# Prevalence of Ascariasis and Amebiasis in Cherokee Indian School Children

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PHYSICIANS at the Public Health Service Indian Hospital on the Cherokee North Carolina Indian Reservation diagnosed several cases of severe clinical ascariasis in children in 1964-65 and recorded the death of a child caused by what was believed to be an overwhelming infection with *Ascaris lumbricoides*. A preliminary survey in one part of the reservation in 1963 indicated that 50 percent of the children were infected with *Ascaris* worms.

To determine the prevalence of the roundworm and other intestinal parasites in the Cherokee population, a collaborative study in 1965 between physicians at the hospital and the Parasitology Section of the National Communicable Disease Center, Public Health Service, was initiated. Because of logistical problems in obtaining specimens from persons living in the

mountainous area of the reservation, it was decided to examine stool specimens from children attending the elementary school close to the hospital. Moreover, the 655 children in the elementary school would represent a sample of the approximately 5,000 residents of the reservation. More important, such examinations would indicate the prevalence of intestinal parasites in the group most likely to be affected by any species of clinical importance.

## Materials and Methods

One-half pint waxed cardboard cartons labeled with each child's name were distributed to the children at the elementary school along with instructions to bring a stool specimen, preferably a morning one, on the following day. The cartons were collected at the school from each pupil in the morning, and all specimens were processed within 6 hours. The stools were preserved in 10 percent formalin; those which were watery, loose, or soft were also placed in polyvinyl alcohol (PVA) fixative (1). When all the stools of a particular day had been preserved, a direct saline and iodine wet mount of the formalinized sediment was examined, as well as a subsequent formalin ether (FE) concentration (2). The stools preserved in PVA were stained with Wheatley's trichrome (3) and examined for protozoan trophozoites.

To determine the correlation between seropositivity and etiological results, serum speci-

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mens were collected from as many children as possible and tested for antibody to *Entamoeba histolytica* and *A. lumbricoides*. Results of the *Ascaris* serology will be reported elsewhere. The amebiasis serology was conducted by using tanned, sensitized sheep red blood cells in the indirect hemagglutination test of Kessel and associates (4) as modified by Milgram and associates (5). The tests were run in microtitration plates, and titers of 1:128 or greater were considered positive. The antigen employed in the test was a sonicated, lyophilized extract of *E. histolytica* strain DKB, grown with *Mycoplasma* organisms. Stock cultures of the amebae had been furnished by Dr. William K. Lewis, University of California, Los Angeles.

## Results

Stools were submitted by 631 children (302 boys and 329 girls), representing 96 percent of the 655 students enrolled in the elementary school.

**Helminths.** The impression of the hospital physicians that *Ascaris* infections were common in the school population was borne out by the observation that 49 percent of the children were infected with the worms. The overall prevalence of *A. lumbricoides* and the other intestinal parasites is shown in table 1. Among the other helminths, only *Trichuris trichiura* was present to a considerable degree (38 percent). All the *Ascaris* and *Trichuris* eggs were detected in the direct wet mount or FE concentrate with the exception of a single infertile *Ascaris* egg found in the PVA stained slide of a stool specimen from an 11-year-old boy. A greater number of hookworm infections would have been detected if a more sensitive technique, such as the culture method of Harada and Mori (6), had been employed. Pinworm infections are not generally detected by stool examinations, but the few cases that were detected are included in the results. Although only 14 children had positive *Enterobius vermicularis* infections, 92 percent were infected with one or more parasites; in only one of the infections caused by *E. vermicularis*, did such eggs represent the sole parasitic stages recovered.

**Protozoa.** Sixty-seven (11 percent) of the children were found to be passing cysts, trophozoites of *E. histolytica*, or both. One-third or

**Table 1. Prevalence of intestinal parasites in stool specimens from 631 Cherokee Indian elementary school children**

Parasite	Specimens positive	
	Number	Percent
<b>Helminths:</b>		
<i>Ascaris lumbricoides</i> .....	312	49
<i>Trichuris trichiura</i> .....	240	38
Hookworm.....	19	3
<i>Trichostrongylus</i> species.....	1	( <sup>1</sup> )
<i>Enterobius vermicularis</i> .....	14	2
<b>Protozoa:</b>		
<i>Entamoeba histolytica</i> .....	67	11
<i>Entamoeba hartmanni</i> .....	220	35
<i>Entamoeba coli</i> .....	251	40
<i>Endolimax nana</i> .....	289	46
<i>Iodamoeba bütschlii</i> .....	34	5
<i>Giardia lamblia</i> .....	59	9
<i>Dientamoeba fragilis</i> .....	68	11
<i>Trichomonas hominis</i> .....	72	11
<i>Chilomastix mesnili</i> .....	19	3
Unidentified protozoa.....	7	1
1 or more parasites.....	579	92
No parasites found.....	52	8

<sup>1</sup> 0.2 percent.

more of the children harbored the commensal amebae—*Entamoeba hartmanni*, *Entamoeba coli*, and *Endolimax nana*. Protozoa were detected in seven stool specimens, but specific identification was not possible because of poor fixation or paucity of organisms. No parasitic organisms were detected in 52 (8 percent) of the 631 stools examined.

The prevalence of six parasite species in boys and girls is compared in table 2. Analysis of the data indicated no difference by sex in parasitization by *Ascaris* (154 males, 158 females) or by *Trichuris* (112 males, 128 females). The number of hookworm infections was too small for adequate comparison (eight males, 11 females). In the stools of 37 boys and 22 girls, the pathogenic, or potentially pathogenic, protozoan *Giardia lamblia* (7) was found. Specimens from 37 boys and 31 girls were positive for *Dientamoeba fragilis*, a parasite with questionable capacity to cause symptoms (8). Although the prevalence of *E. histolytica*, *G. lamblia*, and *D. fragilis* appears greater in males than in females, an analysis of parasitization by sex was not possible. Stools from both sexes were examined after FE concentration, but there was a disproportionately larger number of PVA stained

slides from boys (62) than from girls (50). The data, therefore, are biased in favor of stool specimens from boys.

The prevalence of the parasites in the children by school grade is presented in tables 3-5. Even though *Ascaris* and *Trichuris* were more prevalent in the lower grades (table 3), at least one-third of the children in the higher grades

also harbored roundworms and whipworms. Results in the category "no parasites found" include examinations for helminths and protozoa. The largest number of stools with no parasites found were from children in the fourth, sixth, and seventh grades, a result indicating no specific pattern and certainly no evidence of a diminution of infection in the older children.

**Table 2. Prevalence of selected parasites in stool specimens from 631 Cherokee Indian elementary school children, by sex**

Parasite	All children		Boys		Girls	
	Number	Percent	Number	Percent	Number	Percent
<i>Ascaris lumbricoides</i> .....	312	49	154	49	158	51
<i>Trichuris trichiura</i> .....	240	38	112	47	128	53
Hookworm.....	19	3	8	42	11	58
<i>Entamoeba histolytica</i> .....	67	11	39	58	28	42
<i>Giardia lamblia</i> .....	59	9	37	63	22	37
<i>Dientamoeba fragilis</i> .....	68	11	37	54	31	46

**Table 3. Percentage of 631 Cherokee Indian elementary school children with helminth parasites, by school grade**

Parasite	School grade								Overall prevalence	
	1	2	3	4	5	6	7	8		
<i>Ascaris lumbricoides</i> .....	57	56	52	51	60	42	37	33	(1)	49
<i>Trichuris trichiura</i> .....	40	51	36	39	42	29	29	30		38
Hookworm.....	0	2	5	1	5	5	4	4		3
<i>Trichostrongylus</i> .....	0	0	0	0	1	0	0	0		
<i>Enterobius vermicularis</i> .....	1	3	5	3	2	2	0	3		2
No parasites found <sup>2</sup> .....	4	8	5	14	4	11	15	7		8
Number of students.....	95	101	64	80	84	62	75	70	-----	

<sup>1</sup> 0.2 percent.

<sup>2</sup> Includes protozoa and helminths.

**Table 4. Percentage of 631 Cherokee Indian elementary school children with protozoan parasites, by school grade**

Parasite	School grade								Overall prevalence
	1	2	3	4	5	6	7	8	
<i>Entamoeba coli</i> .....	40	41	39	34	48	47	33	37	40
<i>Endolimax nana</i> .....	52	37	50	49	51	29	43	56	46
<i>Iodamoeba bütschlii</i> .....	0	5	3	5	7	3	9	11	5
<i>Giardia lamblia</i> .....	14	11	9	6	10	13	6	4	9
<i>Dientamoeba fragilis</i> .....	18	7	8	9	12	11	15	6	11
<i>Chilomastix mesnili</i> .....	5	3	6	0	2	5	1	1	3
<i>Trichomonas hominis</i> .....	9	8	6	12	20	16	8	11	11
Unidentified protozoa.....	0	0	3	0	5	3	3	0	1
Number of students.....	95	101	64	80	84	62	75	70	-----

**Table 5. Percentage of 631 Cherokee Indian elementary school children with *Entamoeba histolytica* and *Entamoeba hartmanni* and the amebic prevalence rate, by school grade**

Parasite	School grade								Overall prevalence
	1	2	3	4	5	6	7	8	
<i>Entamoeba histolytica</i> .....	8	6	9	14	14	8	13	13	11
<i>Entamoeba hartmanni</i> .....	37	26	33	36	38	37	28	51	35
<i>E. histolytica</i> and <i>E. hartmanni</i> combined.....	39	26	34	40	43	38	35	56	39
Amebic prevalence rate.....	91	64	77	69	74	69	64	81	74
Number of students.....	95	101	64	80	84	62	75	70	-----
Amebiasis hemagglutination test positive (percent) <sup>1</sup> .....	2	2	2	1	1	2	4	1	2

<sup>1</sup> Indirect HA titers 1:128 or greater.

The seven species of protozoa in table 4 occurred without any appreciable diminution through the eighth grade. *E. coli* and *E. nana* were more prevalent than the other five species.

*E. histolytica* organisms were found in 67 children (39 boys, 28 girls)—an 11 percent prevalence rate (table 5). Forty-three (64 percent) of the *E. histolytica* infections were diagnosed from cysts found in the FE concentrates; seven (11 percent) were diagnosed from organisms found in both PVA stained slides and FE concentrates; 17 (25 percent) were diagnosed in the PVA stained slide only.

Distribution of the *E. histolytica* throughout the school grades was constant, with more infections in the older children (in the seventh and eighth grades) than in the younger ones (in the first, second, and third grades).

Of particular significance was the observation of an "amebic prevalence rate" (APR) of 74 percent. The APR, first described by Brooke and associates (9), is calculated by considering infections with one or more of the four amebae (*E. histolytica*, *E. coli*, *E. hartmanni*, and *E. nana*) as an "amebic" infection. Since the four organisms have comparable but not identical capabilities of surviving in the environment and are transmitted by ingestion of cystic stages, they are indicative of fecal contamination.

*Amebiasis serology.* Serum specimens were collected from 617 children. Specimens were obtained from all but one of the 67 children whose stools were positive for *E. histolytica*. The following summary of the correlation between etiological and serologic positivity in the 617 children is taken from a table in a previous study by Healy (10).

Test results	Number	Percent
<i>E. histolytica</i> in stools, IHA test positive.....	2	0.3
<i>E. histolytica</i> in stools, IHA test negative.....	64	10.4
No <i>E. histolytica</i> in stools, IHA test positive.....	10	1.6
No <i>E. histolytica</i> in stools, IHA test negative.....	541	87.7
Total.....	617	100.0

Ninety-eight percent of the 617 serums were negative for ameba antibody. Only two (0.3 percent) of the serum specimens were positive with the corresponding *E. histolytica* found in the stool specimens, whereas 10 (1.6 percent) of the serum specimens were positive without demonstration of the amebae in the stool specimens. Organisms recovered in stool specimens from children with positive IHA titers are listed in table 6.

## Discussion

Since our survey was concerned with intestinal parasites in elementary school children, no reference can be made to the extent of parasitism in the population of the Cherokee Indian Reservation. Direct examination of a single stool specimen and examination after FE concentration probably resulted in detection of all of the *Ascaris* infections and the majority of the *Trichuris*. The same cannot be said for the intestinal protozoa. Although all specimens were subjected to direct and FE concentrate examinations, only 112 of the 631 stools were preserved in PVA and subsequently examined after trichrome staining. The possibility of more widespread protozoan infections is suggested by table 7, which shows the parasite prevalence in the siblings of three families.

Eleven reports of parasitism among North American Indians have been published (11-21). The present study was concerned only with elementary school children from 6 to 16 years of age, whereas most other surveys have included all age groups. *Ascaris* eggs were reported in only three other studies (13, 14, 20); in the study

by Fournelle and associates (20), *Ascaris* eggs were found in only one of the 855 stools examined.

A recent survey of intestinal parasitism in the Southeast was conducted by Jeffery and associates (22) in a coastal area of South Carolina in Beaufort County among a rural Negro popula-

**Table 6. Parasites recovered from 12 Cherokee Indian elementary school children with positive titers for amebiasis in the indirect hemagglutination test**

Child's age, sex, and grade	IHA titer	Parasites found
7, F, 1st-----	1:256	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , <i>Entamoeba histolytica</i> , <i>Endolimax nana</i> .
8, M, 1st-----	1:128	<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>Entamoeba hartmanni</i> , <i>E. nana</i> .
8, M, 2d-----	1:256	<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>E. hartmanni</i> , <i>Trichomonas hominis</i> .
7, F, 2d-----	1:128	<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>Entamoeba coli</i> , <i>E. nana</i> .
8, M, 3d-----	1:128	<i>A. lumbricoides</i> .
10, F, 4th-----	1:256	<i>A. lumbricoides</i> , <i>E. coli</i> , <i>E. nana</i> , <i>Iodamoeba bütschlii</i> , <i>T. hominis</i> .
11, M, 5th-----	1:128	<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>E. histolytica</i> , <i>E. coli</i> .
12, M, 6th-----	1:128	Unidentified flagellate.
12, F, 7th-----	1:128	No parasites found.
14, M, 7th-----	1:128	<i>A. lumbricoides</i> , <i>T. trichiura</i> , <i>E. hartmanni</i> .
14, F, 7th-----	1:128	<i>T. trichiura</i> , <i>E. coli</i> .
14, F, 8th-----	1:128	<i>E. coli</i> , <i>E. hartmanni</i> , <i>E. nana</i> .

**Table 7. Occurrence of parasites among Cherokee Indian elementary school children from three family groups**

Sibling's sex and age	Parasites <sup>1</sup>										
	Al	Tt	Eh	Ehart	Ec	En	Ib	Df	Gl	Th	Cm
Family A:											
Boy, 7-----	x	x	x	x	x						
Girl, 8-----	x	x			x		x				x
Girl, 10-----	x	x		x	x	x	x				
Boy, 11-----	x	x	x		x	x	x				x
Boy, 13-----	x	x			x	x	x				
Boy, 14-----				x		x					
Family B:											
Girl, 6-----	x	x	x	x		x		x			
Girl, 8-----	x	x				x		x		x	
Girl, 10-----	x	x			x			x		x	
Boy, 11-----	x	x		x	x						
Girl, 12-----	x	x									
Girl, 15-----	x	x		x			x				
Family C:											
Boy, 6-----	x				x						
Girl, 8-----					x				x		
Girl, 11-----	x			x	x	x			x	x	
Girl, 13-----	x					x		x	x	x	
Girl, 14-----	x			x	x	x		x	x	x	

<sup>1</sup> Key: Al—*Ascaris lumbricoides*  
Tt—*Trichuris trichiura*  
Eh—*Entamoeba histolytica*  
Ehart—*Entamoeba hartmanni*  
Ec—*Entamoeba coli*  
En—*Endolimax nana*

Ib—*Iodamoeba bütschlii*  
Df—*Dientamoeba fragilis*  
Gl—*Giardia lamblia*  
Th—*Trichomonas hominis*  
Cm—*Chilomastix mesnili*.

tion. They examined family units and found the following overall prevalence of parasites from 212 stools: *Ascaris* 64 percent, *Trichuris* 37 percent, *E. histolytica* 1.4 percent, *E. coli* 32 percent, *E. nana* 10 percent, *Iodamoeba bütschlii* 0.5 percent, *Trichomonas hominis* 0.5 percent, *Chilomastix mesnili* 1.4 percent, and *G. lamblia* 8 percent. Prevalence for *E. hartmanni* and *D. fragilis* was not given.

In the age groups comparable to those of the Cherokee school children (6-17 years), the survey of Jeffery and associates showed 60 of 74 children (81 percent) positive for *Ascaris* and 38 of 74 (51 percent) positive for *Trichuris*. There was no analysis of parasitization for the protozoa by age groups. Although the sample studied by Jeffery and associates was smaller, both for all persons infected and for children, these persons from South Carolina had a higher prevalence of *Ascaris* (64 percent overall and 81 percent in children) than did the Cherokee children (49 percent).

When our survey is compared with the one of Jeffery and associates, certain differences are noted. Our survey was conducted among American Indian children in western North Carolina in the Great Smoky Mountains while the survey of Jeffery and associates was conducted among American Negro families in the flat coastal plains of South Carolina.

The similar high prevalence of fecally transmitted intestinal parasites in the two groups, however, emphasizes the important point made by Jeffery and others, that "a high incidence of *Ascaris* in specific groups may not depend so much on the climate or topography, although certain favorable conditions are necessary, as on the particular habits and sanitation of the populations involved."

The survey of the Cherokee Indian children was conducted with three goals in mind. The first was to provide physicians of the Public Health Service Indian Hospital with information on the prevalence of *Ascaris* and other parasites in the school population. As suspected, the prevalence of *Ascaris* infections was high, and the amebic prevalence rate was as high as in some tropical areas.

The second goal was to determine the suitability of serologic tests for ascariasis and amebi-

asis. Evaluation of *Ascaris* serology is in progress. Results of the amebiasis serology enabled us to evaluate the specificity of the indirect hemagglutination (IHA) test for intestinal amebiasis. The results of our evaluation of 617 serum samples taken from infected children indicated no cross reactions with other intestinal parasites. The 617 serums from our survey along with the other serums tested (10) also showed that the IHA test was of little value in asymptomatic intestinal amebiasis. Such results are in keeping with the concept that positive serology is evident only where tissue invasion has occurred, as in amebic dysentery or amebic liver abscess. The serologic results corroborated the experiences of physicians from the Public Health Service Indian Hospital, who have recorded only rare instances of clinical amebiasis in the Cherokee Indian population.

The third, and perhaps most useful goal, was to document the prevalence of fecally transmitted parasites. With the exception of hookworm and pinworm, the presence of intestinal parasites indicates the status of a population's sanitation and personal hygiene. Therefore, data on prevalence from our survey would serve as a basis for judging any changes occurring subsequently as a result of improvements in sanitation, intensified health education, or regimens of drug prophylaxis directed against *Ascaris* or other parasites. For example, the effects of certain sanitary improvements that were being provided the Cherokee Indian Reservation under Public Law 86-121 at the time of our survey might be gauged by comparing the prevalence of *Ascaris* or other parasites that we found with the prevalence observed in a similar survey several years from now. (Public Law 86-121 included provisions for the construction of either a well-built, suitably placed outdoor pit privy or the piping in of water for use with an indoor toilet and with facilities for washing and bathing. The Indian householder was required to contribute a certain amount of labor to initiate the construction of either of the two kinds of sanitary facilities.)

### Summary

Single stool specimens, collected from each of 631 children at the Cherokee Indian Elementary School, Cherokee, N.C., were examined for

intestinal parasites. The organisms identified and their prevalence were as follows: *Ascaris lumbricoides*, 49 percent; *Trichuris trichiura*, 38 percent; hookworm, 3 percent; *Entamoeba histolytica*, 11 percent; *Entamoeba hartmanni*, 35 percent; *Entamoeba coli*, 40 percent; *Endolimax nana*, 46 percent; *Iodamoeba bütschlii*, 5 percent; *Giardia lamblia*, 9 percent; *Dientamoeba fragilis*, 11 percent; *Chilomastix mesnili*, 3 percent, and *Trichomonas hominis*, 11 percent.

Evidence of infection with one or more parasites was found in 92 percent of the children. The amebic prevalence rate, which can be used to measure the extent of ingestion of organisms through fecal contamination, was 74 percent. There was no difference in the prevalence of *A. lumbricoides* or *T. trichiura* between Indian boys and girls. Although there was a slight reduction in the prevalence of some parasites (*A. lumbricoides*, *T. trichiura*, and *G. lamblia*) in children of the higher elementary grades as compared with the lower ones, in many cases an equal or greater number of children in the higher grades were parasitized with *E. histolytica* and *E. hartmanni* as compared with children in the lower grades. In general, the survey revealed a high prevalence of intestinal parasites in children throughout the eight grades of the school.

An indirect hemagglutination (IHA) test for amebiasis was used to detect antibody in the serums of 617 of the children. Results showed no cross reactions with any other intestinal parasites. Only two of the serums (0.3 percent) from children having *E. histolytica* in the stools were positive by IHA; conversely, 10 of the serums (1.6 percent) from children in whom no *E. histolytica* was detected were positive. Results of the indirect hemagglutination test indicated that it was of little value in asymptomatic intestinal amebiasis. They did, however, corroborate the experience of the Public Health Service physicians, who had rarely found cases of clinical amebiasis in the Indian children.

Data obtained in the survey will serve as a basis by which to judge the results of projected activities designed to improve sanitation, intensify health education, and provide drug prophylaxis for ascariasis.

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#### Tearsheet Requests

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## Link Between XYY Syndrome and Criminality Not Clear

A link between the XYY syndrome—an in-born male chromosome abnormality—and criminal behavior is not clearly demonstrated at this time.

This was concluded by a panel of experts at a conference on the XYY syndrome, sponsored by the National Institute of Mental Health's Center for Studies of Crime and Delinquency, held in Chevy Chase, Md., June 19-20, 1969.

Chaired by Dr. Park Gerald of Boston's Children's Hospital, the conferees, including experts in genetics, psychiatry, psychology, sociology, and law, discussed in-depth research, ethical, and social policy issues related to the XYY syndrome.

A major focus was on development of broad and flexible guidelines for research procedures in studies designed to ascertain the true prevalence and frequency of the XYY chromosome constitution in the general population so that future studies can be combined and inter-related more effectively.

Regarding various legal questions which increasingly are being raised, consensus was that presently no definite conclusions can be drawn about the relationship between the presence of the XYY chromosome and deviant, criminal, and violent behavior. The general impression that the XYY chromosome is clearly related to criminal behavior is definitely mis-

leading, according to the specialists. They believe that this impression has created a public concern which is premature, since available research evidence is not adequate to support the assertion.

Dr. Saleem A. Shah, chief of the Center for Studies of Crime and Delinquency, said that a prime reason for the concern of the National Institute of Mental Health in this area is to obtain valid information about the prevalence of persons with the XYY syndrome in the general population, and to undertake careful and systematic research to ascertain the nature of the interactions between such chromosomal anomalies and complex social behavior. The shortage of information about the frequency of such cases makes it difficult to evaluate properly the meaning and significance of the XYY cases which have been found among various criminal populations. Shah expressed concern about the strong pressures that are being brought about in this, as yet, little understood area. Moves to change social policy are already outpacing and are out of touch with the present state of scientific knowledge, he said.

A brief publication summarizing the findings, conclusions, and recommendations of the conference will be available from the Center late in 1969.